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Ultrasound evidence of fetal resorption in the yellow stingray, *Urobatis jamaicensis* (Urotrygonidae)

by

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Résumé. – Preuve par échographie de la résorption embryonnaire chez *Urobatis jamaicenis* (Urotrygonidae).

La technique échographique a permis de documenter la résorption embryonnaire sur trois pastenagues jaunes gestantes, *Urobatis jamaicensis* (Cuvier, 1817), à l'aquarium de John Shedd, Chicago, Illinois, USA. La copulation a eu lieu entre le 30 avril et le 16 mai 2010. La fécondation a été vérifiée par échographie mensuelle jusqu'à ce que la gestation soit confirmée au second et au quatrième mois, et celle-ci suivie durant les trois derniers mois. Au cinquième mois, l'échographie a mis en évidence la présence de tissus embryonnaires dans les utérus, mais n'a pas détecté de fréquence respiratoire. Au sixième mois, l'absence complète de tissu embryonnaire dans les cornes utérines des trois femelles a été confirmée. A notre connaissance, c'est le premier cas de résorption embryonnaire décrit chez *U. jamaicensis*.

Key words. – Urotrygonidae - *Urobatis jamaicensis* - Elasmobranch - Aplacental viviparity - Embryonic resorption.

Various forms of fetal death can occur in both placental and aplacental animals. Fetal resorption may occur in polytocous (i.e. producing many youngs or eggs) species because complete abortion of a pregnancy, due to a single fatality, would cause all other siblings to be aborted simultaneously (Long and Parkes, 1924). During resorption fetal tissue can be absorbed entirely by the mother with no external signs of pregnancy loss, thereby making resorption difficult to document and study.

To our knowledge, fetal resorption has not been recorded in elasmobranchs though fetal mortality has been well documented. Some elasmobranchs, including the silky shark, *Carcharhinus falciformis*, are able to internally sequester fetal mortalities during gestation and still give birth to full-term live pups (Sandoval-Castillo and Villavicencio-Garayzar, 2008). In lamnid sharks, ovatrophy or fetal oophagy on both fertilized and unfertilized eggs has been documented (Gilmore, 1993), while adelphotrophy or intrauterine cannibalism has been documented in other elasmobranchs (Joung and Hsu, 2005). Here we used ultrasound technology to document fetal resorption in three pregnant yellow stingrays, *Urobatis jamaicensis* (Cuvier, 1817), at the John G. Shedd Aquarium, Chicago, Illinois, USA.

MATERIALS AND METHODS

As part of a larger temperature-dependant reproductive study of *U. jamaicensis*, eight females and one male were maintained in two 3 m diameter, 9,085 L reserve tanks at the John G. Shedd Aquarium. In October 2009, sexually mature stingrays (based on follicle production documented when animals were brought to Shedd) of unknown age were collected in the Bahamas near the island of Bimini. Video cameras and red-filtered lights were installed above the tanks to document mating behaviours and copulation events during the day and night hours. Water parameters were maintained at: temperature 21-22°C, salinity 32-34 ppt, ammonia > 0.00 ppm, nitrates > 20 ppm, and pH 8.00-8.20. Their diet varied daily and consisted of chopped shrimp, squid, herring, capelin, smelt, krill, mackerel, and clam mixtures. Animals were offered two percent of their body mass in food with uneaten food removed each day to maintain optimal water quality.

Females were split into two groups of four and the male was allowed to mate with each group of females for three months. During this period, bite marks were noted on females as potential signs of mating and crosschecked with video to confirm copulation. Since ultrasounds were not performed until approximately one month after copulation was observed, dates confirmed from video were used as estimates of the onset of gestation. After the breeding periods the male was moved to a separate tank to give the females reprieve from mating.

Ultrasound examinations, using a Sonosite VET 180PLUS ultrasound machine (Bothell, WA, USA), were conducted monthly until females were confirmed pregnant. Due to time constraints, follow-up ultrasounds were not performed on pregnant females until their last three months of gestation. Gestational months are approximations since all three individuals copulated on different dates. For ultrasonic examinations and morphometric measurements (body mass and disc width), females were captured by dip net and placed into a shallow basket of water, remaining submerged throughout the 10-minute process. No anesthesia was used during examinations and venomous spines were left intact and uncovered.

To maintain the maternal identity of pups, pregnant females were placed in floating nurseries during their estimated sixth month of gestation. The nurseries were 1 m x 1 m x 0.75 m floating baskets constructed of polyvinyl chloride frames surrounded by plastic mesh. The bottom of each nursery was lined with plastic sheeting and sand to allow females to bury.

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RESULTS

The male was introduced to the first group of four females on 12 February 2010 through 17 May 2010. Mating events varied per couple with copulation observed during the day as well as the night lasting from 30 seconds to five minutes. Three of these four females were confirmed pregnant on their two-month ultrasounds, performed on 30 June 2010 (Tab. I). Corresponding mating bite wounds and copulation events were documented on 30 April 2010 (Female 1), 4 May 2010 (Female 2), and 16 May 2010 (Female 3). Female 4 did not exhibit mating bite marks nor was copulation documented on surveillance videos. The male was introduced to the second group of four females on 17 May 2010 through 18 August 2010, though no copulation events were documented on the surveillance videos and no females were successfully impregnated according to ultrasound scans.

Subsequent ultrasounds were performed on the three pregnant females during gestational month four (8 September 2010). Independent fetal movement was confirmed in all three stingrays by visually documenting fetal wing and respiratory activity. Each female was observed carrying one to three pups (Tab. I, Fig. 1) and respiratory counts were taken on at least one of each mother's pups. During the five-month ultrasounds (performed 20 October 2010) independent fetal movement was unverified and respiratory rates could not be detected. There appeared to be fetal tissues within their uterine horns (Fig. 1) however no clear physical structures such as wing or oral cavities could be identified making viability questionable.

Six-month ultrasounds were performed on 3 November 2010 and revealed empty fluid filled uteri as well as ovaries containing multiple follicles (Fig. 1). The exact number of follicles per individual was not recorded during this study due to the difficulty of viewing them inside a three-dimensional organ using two-dimensional imaging. The ovum size could not be documented since ovulated ova were not observed. Upon visual examination females did not appear dorsally bulbous during the second half of their pregnancies nor was a noticeable concave appearance seen at any point in the study, however, both characteristics have been noted during past successful pregnancies at Shedd Aquarium.

Table I. - Fetal counts along with maternal body mass and maternal disc width measurements.

	Female 1		
Date	# Fetuses	Body mass (kg)	Disc width (cm)
30 Jun. 10	1	0.72	20.0
08 Sept. 10	1	0.72	20.0
20 Oct. 10	1?	0.73	19.5
03 Nov. 10	0	0.64	19.5
	Female 2		
30 Jun. 10	2	0.77	20.0
08 Sept. 10	1	0.77	20.0
20 Oct. 10	1?	0.81	20.5
03 Nov. 10	0	0.79	20.0
	Female 3		
30 Jun. 10	3	1.24	26.5
08 Sept. 10	3	1.15	26.5
20 Oct. 10	1?	1.19	26.5
03 Nov. 10	0	1.15	26.0

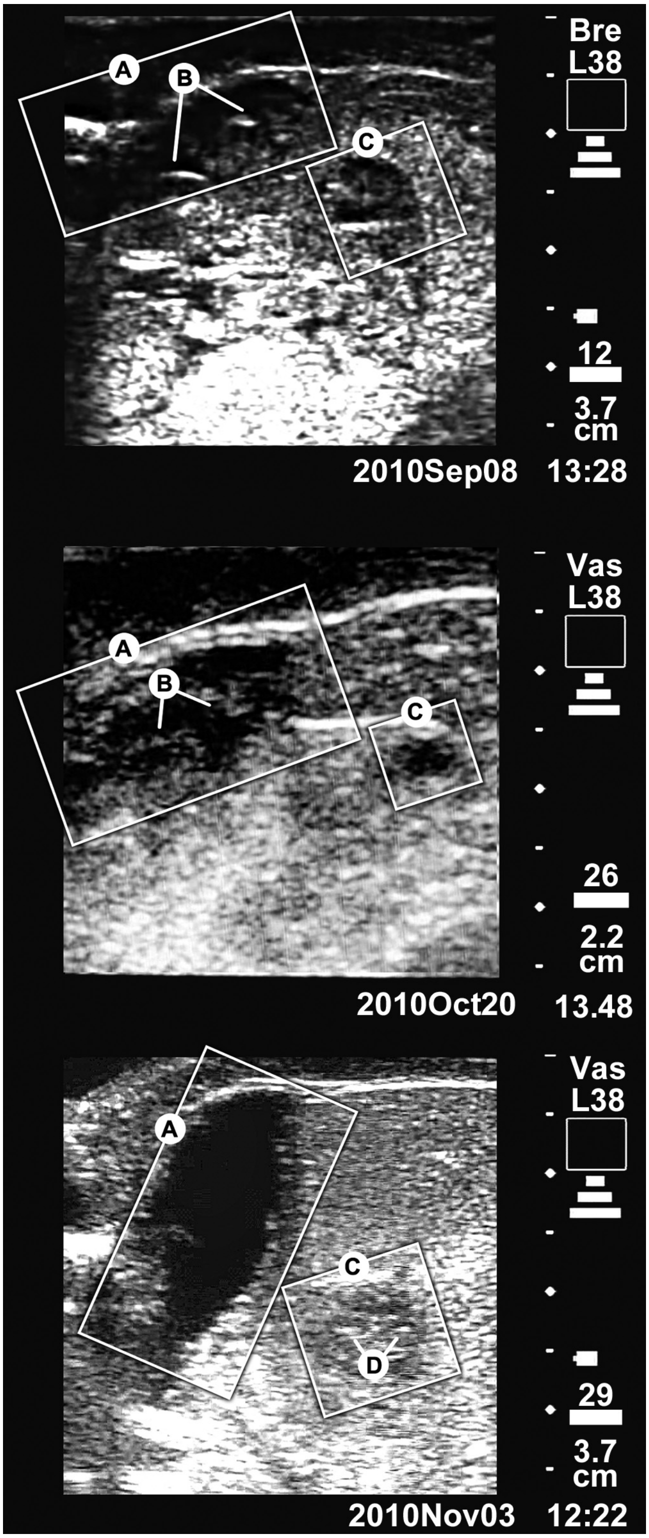

Figure 1. - Representative ultrasound images at four-month post copulation (8 Sep. 2010) before resorption, five-month post copulation (20 Oct. 2010) during resorption, and six-month post copulation (3 Nov. 2010) after resorption. A: Uterine horn; B: Fetus; C: Ovary; D: Follicle.

DISCUSSION

In this study, three females were confirmed pregnant during their two and four-month ultrasounds (Tab. I). The five-month ultrasounds still showed fetal tissues within the uterine horn (Fig. 1); however, mortality was suspected since independent fetal movement could not be confirmed. The six-month ultrasounds confirmed empty uterine horns on all three previously pregnant females and complete absence of any fetal tissues (Tab. I, Fig. 1). In the time between these ultrasounds there was no evidence of stillborn discharge or maternal cannibalism.

Since these proposed resorption events occurred concurrently in all three pregnant females, it is possible that they were the result of a common catalyst. It has been suggested that fetal resorption results from stressors including suboptimal environments or inadequate nutrient availability (Blackburn, 1999). A possible environmental stressor here includes the females being retained in floating nurseries during the end of their gestation period. We do not, however, believe this to be a factor because the nurseries were used successfully in the past at Shedd Aquarium without adverse effects. In fact, in 2009, Female 3 successfully gave birth to three pups in a floating nursery at a mean temperature of 26˚C with a gestation length of approximately seven months. Since the animals were fed to satiation, we do not suspect nutritional factors influenced the resorption events. Instead, environmental temperatures may have influenced resorption in this study.

The females in this study were housed at 21.5˚C, which is considerably cooler than the 26˚C that they have been successfully reared in at Shedd Aquarium. *Urobatis jamaicensis* have been documented to reproduce biannually with peaks in parturition noted in July and December off the coast of southeast Florida (Fahy *et al.*, 2007). Even though temperatures in the wild may decline below 21.5˚C seasonally the prolonged effect of cool temperatures during *U. jamaicensis* pregnancy has not been documented prior to this study. We suspect that the continuous exposure to low temperatures during gestation may have created a thermal environment detrimental to pup survival.

It is common for elasmobranchs to seek environmental conditions that benefit their offspring. The Australian sharpnose shark, *Rhizoprionodon taylori*, mates January through February. Females, however, are suspected to exhibit embryonic diapause for the following six months before fertilizing the ovum, thus allowing coordination of their pregnancy with optimal seasonal temperatures (Simpfendorfer, 1992). Embryonic diapause is not specific to sharks as this phenomenon is also suspected in *Trygonoptera imitata* a round ray found in the coastal waters of Australia (Trinnie *et al.,* 2009). Behavioural thermoregulation is conducted by poikilotherms to regulate physiological processes. This behaviour is seen in the Atlantic stingray, *Dasyatis sabina,* which move to cooler waters after feeding to slow digestion and extend time between meals (Wallman and Bennet, 2006). It is unknown if *U. jamaicensis* uses environmental temperatures behaviourally for reproductive advantages. However pregnant female *U. halleri*, the closest relative of *U. jamaicensis,* are known to congregate in the warm shallow waters of southern California (Mull *et al.,* 2010). The resulting effect of temperature on *U. halleri* offspring was not the focus of the Mull *et al.* (2010) study although their behaviour suggests some type of thermoregulatory advantage.

In our study, *U. jamaicensis* were held at a constant temperature and did not have the ability to seek warmer waters. Since *U. jamaicensis* are known to be biannual seasonal breeders, they may require temperature cues for proper embryonic development. Therefore the absence of optimum environmental cues may have ultimately resulted in resorption. The data presented here should be corroborated with further study, and considered when determining the requirements for optimal breeding conditions of *U. jamaicensis*.

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